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Published in:
Skin Pharmacology and Physiology

DOI:
[10.1159/000497104](https://doi.org/10.1159/000497104)

Publication date:
2019

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Document Version
Publisher's PDF, also known as Version of record

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Groten, K., Marini, A., Grether-Beck, S., Jaenicke, T., Ibbotson, S., Moseley, H., Ferguson, J., & Krutmann, J. (2019). Tomato phytonutrients balance UV response: Results from a double blind, randomized, placebo controlled study. *Skin Pharmacology and Physiology*, 32(2), 101-108. <https://doi.org/10.1159/000497104>

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Tomato Phytonutrients Balance UV Response: Results from a Double-Blind, Randomized, Placebo-Controlled Study

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Keywords

Carotenoids · Oral photoprotection · Erythema formation · UVB · Double-blind, randomized, placebo-controlled study

Abstract

Background: Our previous double-blinded, placebo-controlled cross-over study indicated that a nutritional supplement named lycopene-rich tomato nutrient complex (TNC) can protect from UVA1-induced (340–400 nm) and UVA- (320–400 nm)/UVB-induced (280–320 nm) upregulation of molecular markers associated with oxidative stress, inflammation, and ageing. **Objectives:** in the current double-blind, randomized, placebo-controlled multicenter study, we analyze whether a similar, synergistic carotenoid-rich TNC can protect from broadband UVB-induced threshold erythema formation assessed as increase in minimal erythmal dose (MED) reading, the intensity of erythema formation, and the upregulation of molecular markers associated with inflammation and immunosuppression, and whether this correlates with carotenoid blood levels. **Methods:** One hundred and forty-nine healthy volunteers were randomized to two groups and subjected to a 5-week washout phase, followed

by a 12-week treatment phase receiving either 15 mg lycopene, 5.8 mg phytoene and phytofluene, 0.8 mg β -carotene, 5.6 mg tocopherols from tomato extract, and 4 mg carnosic acid from rosemary extract per day or placebo made from medium-chain triglycerides. At the end of each phase, MED determination, UVB irradiation, chromametry, biopsies, and blood samples were undertaken. **Results:** The active supplement was well tolerated. Interestingly, no significant difference was seen in the MED between the active-supplement and placebo groups, as determined by visual grading by expert assessors. Of note, the carotenoid-containing supplement significantly protected against UVB-induced erythema formation measured as Δa^* after the intervention minus Δa^* after the washout phase as compared to the placebo. Moreover, intake of the active supplement significantly protected against UVB-induced upregulation of IL6 and TNF α as compared with the intake of placebo. Lastly, carotenoid plasma levels were significantly increased. **Conclusion:** This well-tolerated carotenoid-containing supplement significantly protected against UVB-induced erythema formation and upregulation of proinflammatory cytokines in healthy volunteers.

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Introduction

The concept of oral photoprotection by antioxidant micronutrients gained very popular over the last decades [1, 2]. In case of topical sunscreens, the sun protection factor is a simple and noninvasive tool to quantify the photoprotective activity. The latter is defined as a quotient of the minimal erythral dose (MED) under treatment divided by the MED without intervention representing a measure for acute deleterious effects of UV irradiation [3]. An increased MED after intervention with a nutritional supplement can also therefore be used as an indicator of photoprotection in studies of potential orally ingested photoprotectants. A special focus was on β -carotene, which had been shown in a meta-analysis to provide significant protection against UV-induced erythema if taken at least over a period of 10 weeks [4]. Safety concerns after long-term intake of β -carotene at non-physiological levels arose when adverse effects on the incidence of lung cancer in smokers and workers exposed to asbestos were found [5]. To circumvent this issue, other carotenoids such as lycopene or lutein came into focus. Recently, a double-blinded, placebo-controlled crossover study has shown that both these ingredients provided significant protection against UVA- or UVA/UVB-induced upregulation of UV-inducible markers such as heme oxygenase 1, intercellular adhesion molecule 1, and matrix-metalloproteinase 1 [6]. Some limitations of that study were (i) the relatively low number of volunteers included and (ii) the lack of showing an effect on the physiological erythral skin response after intervention with the active supplement. In order to complement and strengthen the results of that study, we therefore performed a double-blind, randomized, placebo-controlled, multicenter follow-up study to assess as primary objective the effect of carotenoid-rich tomato nutrient complex (TNC; containing and rosemary extract) on the acute UV radiation-mediated erythral skin response; i.e., (i) MED and (ii) intensity of erythema, compared with the placebo cohort following 12 weeks of twice daily oral supplement. As secondary objective, we wanted to evaluate the effect of carotenoid-rich TNC compared to that of placebo after 12 weeks of b.i.d. (twice a day) supplementation on skin biopsy biomarkers indicative for immunosuppression or inflammation. Analysis of UV-induced gene expression was very helpful to address photodamage to human skin in vivo in the previous study [6]. With regards to UVB, IL1 α was shown to be upregulated in keratinocytes in vitro and in vivo [7, 8]. IL10 was shown to be induced in keratinocytes in vitro and in human skin in

vivo, where it exerts profound immunosuppressive activities [9]. Proinflammatory cytokines such as TNF α and IL6 are induced upon UVB treatment in keratinocytes and in humans on mRNA and protein level [10, 11]. For a detailed review on UVB-induced cytokine production by keratinocytes or Langerhans cells, see [12]. The listed proinflammatory cytokines act in a cascade fashion to induce inflammation with initial release by keratinocytes or inflammatory cells in the skin and subsequent synergizing with UV-irradiated keratinocytes to further increase their cytokine production finally affecting local and systemic immunosurveillance [12, 13].

Materials and Methods

Materials

Carotenoid-rich TNC soft gel capsules contained 7.5 mg lycopene, 2.9 mg phytoene and phytofluene, 0.4 mg β -carotene, 2.8 mg tocopherols from tomato extract, and 2 mg carnosic acid from rosemary extract per capsule (2 capsules per daily dose). The placebo was packaged in an identically looking soft gel capsule and consisted of medium-chain triglycerides. Both treatments were obtained from Lycored Ltd., Be'er Sheva, Israel.

Study Design

This study was approved by the local Ethics Committees of (i) the Heinrich Heine University, Düsseldorf, Germany (reference No. 4194), and (ii) the University of Dundee, Scotland, UK (No. SC015096). It was conducted at the IUF – Leibniz-Research Institute for Environmental Medicine, Düsseldorf, Germany, and at the Photobiology Unit, Department of Dermatology, Ninewells Hospital and Medical School, Dundee, UK, according to the ethical rules stated in the principles of the Declaration of Helsinki and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. We conducted a randomized, double-blind, placebo-controlled, parallel-group, multicenter clinical trial in which each subject was randomized to receive carotenoid-rich TNC or placebo for a period of 12 weeks in a parallel-group design. A total of 149 subjects (female or male) were enrolled into the study, and 145 subjects completed the trial as planned. The treatment groups were stratified based on the age category, gender, and smoking to ensure equal distribution of such population within the two treatment arms (Table 1). The group taking the active supplement included 75 volunteers (56 female, 19 male), and the group taking placebo consisted of 74 volunteers (59 female, 15 male). The age distribution did not differ in both arms of the study (mean ages were 40.9 and 40.9 years). The age of the volunteers ranged from 20 to 50 years. Current smokers were very rare; in the carotenoid-rich TNC group, there were 5 smokers (7%), and in the placebo group 3 (4%). For ethical reasons, only two-thirds of the volunteers were asked for biopsies to be analyzed for expression of molecular markers. The subjects enrolled were distributed according to an online block randomization service by the sponsor; block size was 2, allocation ratio 1:1. Volunteers and investigators were unaware of the treatment.

Table 1. Baseline demographics and characteristics

	Carotenoid-rich TNC	Placebo
Participants, <i>n</i>	75	74
Females, <i>n</i> (%)	56 (75)	59 (80)
Males, <i>n</i> (%)	19 (25)	15 (20)
Mean age (SD), years	40.9 (10.3)	40.9 (10.1)
Mean BMI (SD)	25.8 (2.8)	25.3 (3.0)
Mean initial lycopene level, nM	676	710
Median initial lycopene level, nM	646	670
Mean initial phytofluene level, nM	65	80
Median initial phytofluene level, nM	51	55
Mean initial phytoene level, nM	54	46
Median initial phytoene level, nM	50	29

All individuals were of good general health with a body mass index ≤ 30 . Their Fitzpatrick skin type was type I–II. With regards to eating habits, a normal diet and the willingness to follow a lycopene- and antioxidant-restricted diet was requested. Exclusion criteria were sunbed use and pregnancy. The study was conducted between January 2014 and September 2016.

Determination

The MED was determined with a dermalight® 80 MED tester (Dr. Hönle, Gilching, Germany) in the broadband UVB range on visits 2 and 7 on both sides of the buttock by visual grading undertaken by expert assessors. The dermalight 80 MED tester serves for the definition of the MED according to the choice of the light source and skin type. The device contains a foil with 10 different test fields. The varying dosage in the test fields is caused by varying sizes of outlets and therefore varying UV irradiation.

Chromametry

MED was read 24 h after MED testing. Directly after the MED reading, a dose of 1.25 MED was applied on an additional field (12 × 12 mm) of the buttock for skin color determination by chromametry (Chromameter CR400; Konica Minolta Sensing Europe B.V., Bremen, Germany) and for taking biopsies with a dermalight 80 MED tester (Dr. Hönle). The erythema formation was measured as change of skin color a^* , which reflects the redness of the skin according to the Commission Internationale de l'Eclairage, as evaluated in [14]. We followed the recommended guidelines for skin color determination [15]. In this regard, color is expressed in a device-independent, three-dimensional color space reflected by the variables L^* , b^* , and a^* . The latter a^* values represent green in the negative direction and red in the positive direction. The dose of 1.25 MED was selected according to prior experience [16].

Assessment of Blood Samples

Blood samples for carotenoid determination were collected after a 5-week washout phase, at randomization, after 4 weeks of treatment, and at the end of the study in heparinized tubes and immediately centrifuged. The plasma was stored at -80°C until analysis for carotenoids such as lycopene, α -, and β -carotene by

high-performance liquid chromatography, as previously described [6, 17, 18]. In addition, the colorless carotenoids phytofluene and phytoene were measured because these precursors of carotenoids absorb radiation in the UV range [19, 20].

Assessment of Gene Expression

Biopsies were taken 24 h after chromametry using 1.25 MED at visits 4 and 8 (Fig. 1b), snap frozen in liquid nitrogen and stored at -80°C until further analysis as, previously described [21–23]. For the evaluation of UV-induced gene expression, the $2^{(-\Delta\Delta C(t))}$ method is used [24]. Gene expression is indicated as x-fold induction versus an unirradiated control. Primer pairs were as follows:

IL1 α (forward 5'-TGTATGGACTGCCCAAGATGAA-3', reverse 5'-ACTACCTGTGATGGTTTGGGTATC-3', NM_000575.4) [25]; IL6 (forward 5'-CCTCGAGCCACCGGGAA-CG-3', reverse 5'-AA-CTGGACCGAAGGCG-CTTGTG-3', NM_000600.4) [26]; IL10 (forward 5'-AAGACCCAGACATC-AA-GGCG-3', reverse 5'-AATCGATGACAGCGCCGTAG-3', NM_000572.3) [27]; TNF α (forward 5'-GGAGAAGGGTG-ACC-GACTCA-3', reverse 5'-TGCCCAGACTCGGCAAAG-3', NM_000594.3) [28]; and 18S rRNA as housekeeping gene (forward 5'-GCCGCTAGAGGTGAAATTCTTG-3', reverse 5'-CATTCTTG-GCAATGCTTTTCG-3', X03205.1) [29].

Sample Size and Statistical Evaluation

Sample size calculation was based on previously conducted studies by Lycored employing different carotenoid-rich formulations (Lyc-O-Mato and Lyc-O-Guard drink) for the primary endpoint “protection against ultraviolet (UV) light-induced erythema,” such as [19, 30, 31]. The sample size corresponds to an effect size difference at 12 weeks between the two groups, with a power of 80% and a drop-out rate of 10%.

Data management and randomization were done by Medistat Ltd. (Tel-Aviv, Israel). Additional studies were performed in-house with the help of SigmaPlot (version 14.0). Normality of the data was tested using the Shapiro-Wilk test. For comparison of significant differences, the Kruskal-Wallis one-way ANOVA on ranks, *t* test, or Mann-Whitney rank sum tests were performed.

Results and Discussion

For the active arm of the study, 75 volunteers were recruited, and 71 completed the study. For the placebo arm, all 74 recruited volunteers also finished the trial (Fig. 1a).

The treatment was safe and well tolerated, and no serious adverse events were observed within the two study populations. Only one severe adverse event diagnosed as pityriasis rosea occurred in the group with carotenoid-rich TNC intake. In addition, one volunteer in the same group discontinued the study due to mild eczema. Compliance was good in both arms, which was reflected by the carotenoid levels determined at the end of the 5-week washout phase (visit 2), after 4 weeks of intake (visit 5), and at the end of the 12 weeks of intake (visit 8) (Fig. 1b, 2a–c) from blood samples. In detail, after 5 weeks of diet

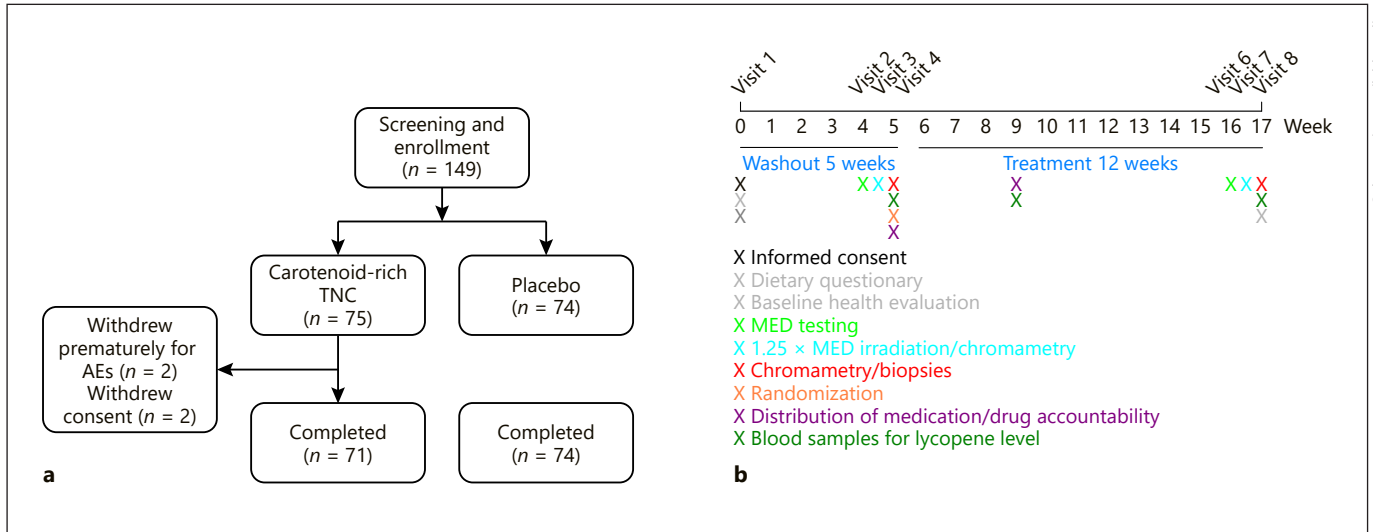


Fig. 1. Design of the study. **a** Overall trial design giving the number of volunteers per protocol and the corresponding dropouts. AEs, adverse events. **b** Details for both arms of the double-blind, randomized, placebo-controlled, multicenter study as performed in each of the centers. Shown are only those visits at which data were collected.

restriction, the starting median (25th percentile–75th percentile) lycopene level was 670 nM (480–919) for the group to take the placebo, and 646 nM (488–890) for the group to take the carotenoid-rich TNC. During the supplementation phase, the lycopene level significantly increased in the carotenoid-rich TNC-taking group to a median of 1,220 nM (832–1,520) and reached a final median level of 1,153 nM (847–1,551), whereas no such increase was seen in the placebo group. There, median levels of 690 nM (472–1,017) after 4 weeks and 720 nM (445–1,040) after 12 weeks were detected.

Similarly, the colorless carotenoid precursor phytofluene significantly increased in the carotenoid-rich TNC arm over time. Median phytofluene was 51 nM (18–94) at the end of the washout phase and increased to 178 nM (79–329) and to 156 nM (66–390) during the supplementation phase in the active arm. In contrast, in the placebo part of the study, phytofluene was 55 nM (18–108) in the depletion phase, which did not significantly change during the intake phase to 67 nM (20–117) and 55 nM (20–108) (Fig. 2b).

Finally, the colorless carotenoid precursor phytoene also significantly increased during the intake phase of the active arm (Fig. 2c). Median phytoene was 50 nM (18–90) at the end of the washout in the group supposed to take the active supplement and increased to 90 nM (78–174) and to 90 nM (66–154) over the supplementation phase.

In the placebo group, the initial median phytoene was 29 nM (16–90). In the supplementation phase, these data did not change and were 52 nM (18–90) and 38 nM (18–90).

Whether the determination of the nutritional supplements such as lycopene from blood samples is a good indicator for compliance and/or bioavailability should be reconsidered because a significant increase in the corresponding plasma levels not always correlates to a significant photoprotection [19]. In this regard, it may be interesting that cutaneous lycopene can also be determined noninvasively by Raman spectroscopy [32], where gender-related differences in basal carotene and lycopene levels have recently been observed.

The first primary outcome, the MED reading, was identical in both intended treatment groups, given as a median (min, max) of 0.080 (0.035, 0.125) after the washout phase. After supplementation with carotenoid-rich TNC, the MED did not change significantly; we observed a median (min, max) of 0.080 (0.035, 0.147; data not shown). This negative result is in line with observations obtained in a human intervention study over 12 weeks, where the MED was compared in a group of 9 females with skin type II receiving 55 g tomato corresponding to 16 mg lycopene in tomato paste to a control group ($n = 8$) taking olive oil [33] and in a comparative 10-week study, where 10 females consumed either a pill or tomato paste corresponding to 16 mg lycopene [34]. In this re-

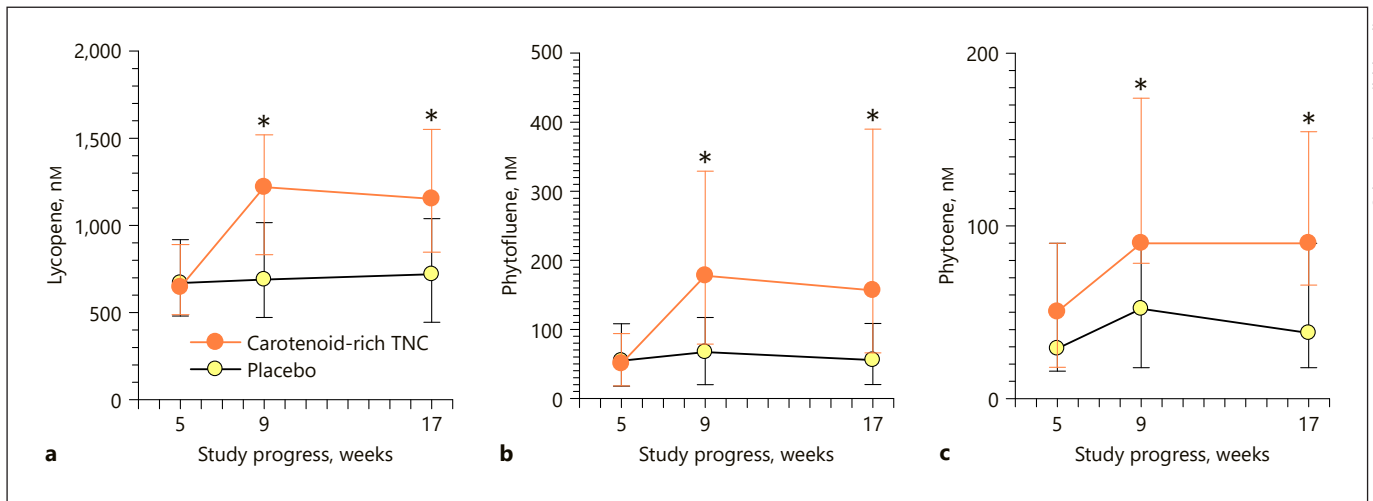


Fig. 2. Compliance reflected by carotenoid blood levels. Lycopene (a) phytofluene (b), and phytoene (c) content in blood samples taken at the indicated time points was determined as described in Materials and Methods. Given are medians, 75th and 25th percentiles from $n = 71$ volunteers taking carotenoid-rich TNC and $n =$

74 volunteers taking placebo. Significance was determined by Kruskal-Wallis one-way ANOVA on ranks (Dunn's) for each time point compared to the starting level at week 5, * $p < 0.05$ versus week 5 (beginning of supplementation).

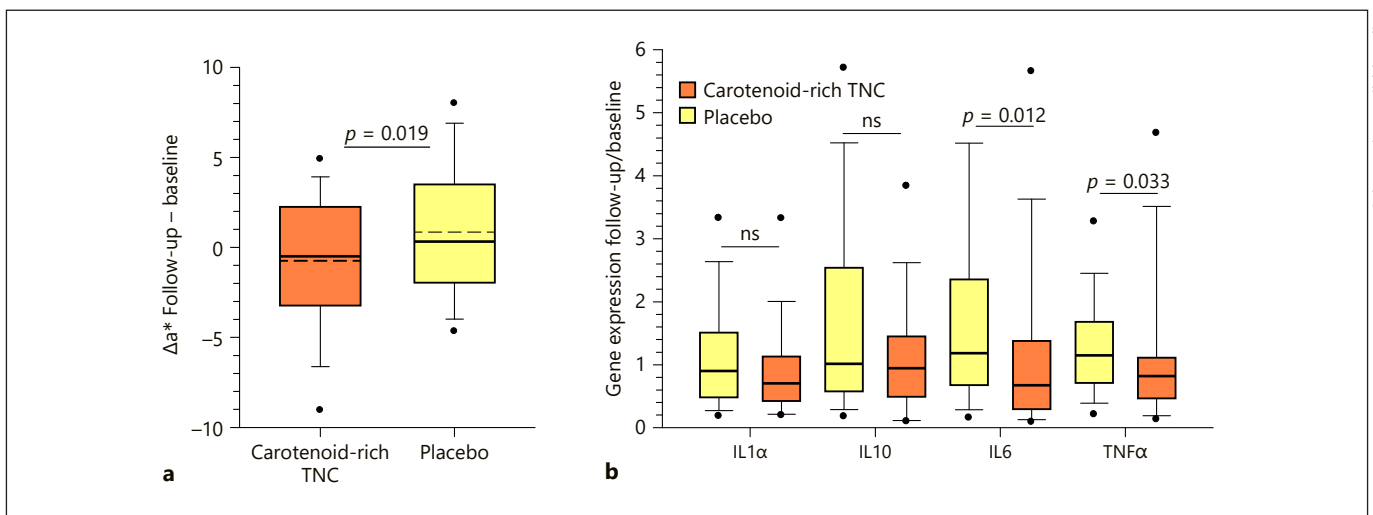


Fig. 3. Effect of carotenoid-rich TNC on erythema formation and gene expression. **a** Change of erythema (Δa^*) formation was determined by chromametry, as described in Materials and Methods, from $n = 71$ volunteers taking carotenoid-rich TNC and $n = 74$ volunteers taking placebo. Δa^* is defined as the difference between erythema development levels at 24 h following UV irradiation after supplementation (visit 8) and erythema development levels at 24 h following UV irradiation before supplementation (visit 4). Given are the differences by box plots with medians (solid line) and means (dashed line), dots represent outliers, and error bars represent the 95th and 5th percentiles. Significance between the treat-

ment groups was determined by the t test; $p < 0.05$ and as indicated. **b** Gene expression analysis presented as quotient of gene induction after 12 weeks of supplementation with carotenoid-rich TNC or placebo (visit 8) divided by gene induction before supplementation (visit 4) from $n = 46$ volunteers taking carotenoid-rich TNC and $n = 48$ volunteers taking placebo. Given are the quotients as box plots with medians; dots represent outliers, and error bars represent the 95th and 5th percentiles. Significance between the treatment groups was determined by the Mann-Whitney rank sum test; $p < 0.05$ and as indicated. ns, not significant.

gard, we must keep in mind that the MED was determined by expert grading in all three studies. Interestingly, Rizwan et al. [33] nevertheless observed a significant increase in the erythema index D_{30} in the tomato paste-consuming group employing a reflectance instrument as compared to the start conditions.

The second primary outcome was the change of erythema Δa^* determined chromametrically (Fig. 3a). Here, we observed a significant difference between the two treatment groups ($n = 71$ for carotenoid-rich TNC, and $n = 74$ for placebo). As expected, the change of erythema a^* after supplementation with carotenoid-rich TNC decreased less than after supplementation with placebo. This can be seen when we use the difference Δa^* of the follow-up minus Δa^* of the baseline. In case of the carotenoid-rich TNC, the response under treatment is lower, and we get a difference below zero. In case of the placebo, the erythema responses after supplementation are not lower, and therefore the difference (follow-up minus baseline) is not below zero. These results corroborate a previous study where a group of nine volunteers eating tomato paste corresponding to 16 mg lycopene, 0.5 mg β -carotene, and 0.1 mg lutein over a 10-week period presented with a significantly decreased Δa^* 24 h after 1.25 MED, while the placebo group of ten people having 10 g of olive oil did not [35]. Similarly, comparing the efficacy of a 12-week intake of either synthetic lycopene, a tomato extract (Lyc-o-Mato), or a drink containing solubilized Lyc-o-Mato (Lyc-o-Guard-Drink) in a parallel-group design ($n = 36$) indicated a significantly decreased Δa^* 24 h after a 1.25 MED irradiation in the groups taking approximately 10 mg/day natural lycopene [19].

The difference to MED determination where we failed in finding protection by the active supplement may be explained by (i) a higher UV dose applied (1.25 MED instead of 1 MED) and by (ii) a higher sensitivity due to an objective detection device, as already shown by Rizwan et al. [33].

A change of skin color, e.g., skin tanning evaluated as ΔL^* or ΔITA , was not observed during the study, no matter whether analyzing the whole population or stratified according to seasonal enrollment. The effect of carotene intake on skin color is still under debate because contradicting results were observed from having an impact only on skin yellowness b^* [36, 37] and or redness a^* [38].

The lycopene-rich TNC assessed in the current study contained 15 mg lycopene, 5.8 mg phytoene and phytofluene, 0.8 mg β -carotene, 5.6 mg tocopherols from tomato extract, and 4 mg carnosic acid from rosemary extract per day because previous in vitro studies/animal

studies indicated a synergistic inhibition of LPS-induced NO production, TNF α -, superoxide and PGE $_2$ release in macrophages and in a mouse model of peritonitis by lycopene, β -carotene, and the phenolic carnosic acid [39]. Similarly, synergistic inhibition of oxidative stress in a mouse tumor model [40] or inhibition of androgen signaling and PSA secretion by lycopene and tocopherol [41] was determined. In addition, the synergistic effect of a TNC and carnosic acid (from rosemary) has recently been observed on inhibition of UVB-induced IL6 secretion in keratinocytes as compared to the single interventions (Calniquer et al., unpublished data).

As secondary outcome, we determined UV-induced gene upregulation of markers associated with immunosuppression or inflammation in biopsies taken at visits 4 and 8. For ethical reasons, we did not take biopsies from all volunteers. Similarly, as for the erythema data Δa^* (Fig. 2b), we further processed the relative gene expression data by building a quotient of induction after supplementation divided by induction before supplementation (Fig. 2c). For the markers IL-1 α and IL10, we did not see a significant improvement by supplementation with carotenoid-rich TNC. In contrast, we saw a significant decrease in the UV response after carotenoid-rich TNC intake as compared to the placebo treatment. Assessment of molecular markers might foster the sensitivity of such studies as shown by a significantly decreased expression of UV-induced matrix metalloproteinase 1 postsupplementation in the lycopene-taking group as compared to the olive oil-taking group [33] or in our previous study [6].

Finally, it should be noted that the daily amount of lycopene used in the current study corresponds to 52 g canned tomato paste and that of β -carotene to 90 g [42]. The amount of vitamin E would be obtained by a daily intake of 130 g of canned tomato paste [42].

Conclusion

Carotenoid-rich TNC is a safe and well-tolerated nutritional supplement suited for significant protection from (i) UVB-induced erythema formation and (ii) UVB-induced upregulation of IL6 and TNF α .

Acknowledgement

The technical support of Heidi Brenden and Ingo Felsner in the analysis of the skin biopsies and the help of the study nurses Lydie Weiland and June Gardner is highly appreciated.

Statement of Ethics

Subjects have given their written informed consent. This multicenter study was approved by the local committees of all participating centers. The study was conducted according to the Declaration of Helsinki principles (2013).

Disclosure Statement

Krutmann, Moseley, and Ferguson obtained funding. The other authors have no conflicts of interest to disclose.

Funding Sources

The costs of conducting this study were paid by Lycored Ltd. Be'er Sheva, Israel.

Author Contributions

Drs. Marini, Grether-Beck, Jaenicke, and Krutmann had full access to all data in the study and take the responsibility for the integrity of the data and the accuracy of the data analysis.

All authors contributed to the study concept and design.

Groten, Marini, Ibbotson, Grether-Beck, Jaenicke, Moseley, Ferguson, and Krutmann were responsible for the acquisition, analysis, and interpretation of the data.

Krutmann, Grether-Beck, Marini, and Groten drafted the manuscript.

Ferguson, Moseley, Ibbotson, and Jaenicke made a critical revision of the manuscript for important intellectual content.

Groten, Grether-Beck, Jaenicke, and Marini made the statistical analysis.

All authors provided administrative, technical, or material support.

Krutmann had the supervision of the study.

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